

CANDIDATE'S ANSWER

- a) Nanoemulsion comprising droplets of a botulinum protein conjugated to polyethylene glycol having an average molecular weight of 2000 to 15000 Dalton, said droplets having an average droplet diameter of less than 1000 nm.
- b) Nanoemulsion according to Claim 1, wherein the droplets have a diameter of 500 nm or less.
- c) Nanoemulsion according to Claim 1 or 2, wherein the droplets are all the same size and have a diameter of 100 nm +/- 10 nm.
- d) A method of producing a nanoemulsion comprising:
 - (i) mixing an aqueous phase containing a botulinum protein conjugated to polyethylene glycol having an average molecular weight of 2000 to 15000 Daltons with an oil phase,
 - (ii) exposing the mixture to a pressure of at least 1000 bar for a period of between 30 seconds to 10 minutes.
- e) A method according to Claim 4, wherein the pressure is 1500 to 2000 bar.
- f) A method according to Claim 4 or 5 wherein the oil phase further comprises a surfactant.
- g) A method according to Claim 6, wherein the surfactant is lecithin.
- h) A method according to Claims 4-7 wherein the aqueous phase makes up at least 90 vol. %, and the oil phase makes up at least 10 vol. %, and wherein the weight ratio of surfactant to oil is 2:1.
- i) Cream for topical administration comprising a botulinum protein conjugated to polyethylene glycol having an average molecular weight of 2000 to 15000 Dalton.
- j) Cream according to Claim 9, wherein the botulinum protein conjugate is in the form of a nanoemulsion according to Claims 1-3.
- k) Cream according to Claim 10, further comprising thickeners, colouring agents, fragrances, preservatives, vitamin C, retinol, collagen and/or coenzyme Q.
- l) A method of reducing wrinkles comprising applying a cream according to Claims 9-11 to a person's face.
- m) A method according to Claim 12 wherein the cream is applied at two-monthly intervals.
- n) A method according to Claim 13, wherein the cream is applied at monthly intervals.
- o) A kit comprising:
 - (i) a cream according to Claims 9-11
 - (ii) a pipette for delivery of the cream.

Nanoemulsions and creams for reducing wrinkles

The present invention relates to nanoemulsions and creams comprising a botulinum protein conjugate for reducing the formation of wrinkles, and methods of making said products.

Background

Facial wrinkles are a visible sign of ageing skin. There are various ways of reducing facial wrinkles. These include face creams, facelift surgery and the injection of botulinum proteins such as the well-known BOTOX®.

The injection of the botulinum protein directly into the facial muscle is one of the most effective means of reducing wrinkles. However, the injection must be performed by a qualified person, usually a specialist doctor, and the patient may find the procedure painful. Also, the treatment usually has to be repeated every three to six months because the botulinum protein's effect wears off considerably over time.

D1 describes forms of botulinum protein that are conjugated to a PEG polymer for injection into the skin to reduce wrinkles. D1 describes that known creams containing botulinum protein alone are not effective at reducing deeper wrinkles in skin. D1 discloses that the only way to reduce deep wrinkles is to inject botulinum protein. D1 describes how by conjugating the botulinum protein to a PEG polymer of a medium weight, it is possible to extend the duration of the effect. D1 proposes that nanotechnology may be of use for preparing injectable botulinum in the future. However no disclosure is made of any nanoemulsion compositions. D1 however does not suggest that such conjugates are also suitable for application directly to the skin; nor does it propose creams comprising said conjugates.

D2 is in the field of topical anti-wrinkle compositions, which comprise of the use of a nanoemulsion of botulinum protein for topical administration. D2 describes methods of making nanoemulsions that comprise droplets of botulinum protein in a distribution of droplet sizes ranging from 100-1000nm. D2 does not however describe any nanoemulsions formed from botulinum protein that is conjugated to polymer in the form of PEG, nor does it provide any suggestion that such polymer has the effect of improving the uptake of botulinum protein across the skin.

These prior art treatments are problematic. D1 requires unpleasant injections in order to administer the botulinum protein; whilst the effects of the anti-wrinkle reduction achieved by D2 are poor and only last for two weeks.

There is therefore a need to provide anti-wrinkle treatments that can be applied directly to the skin and which have an improved longer lasting effect.

The nanoemulsions and creams of the present invention provide a solution to the problem by providing a treatment that can be applied topically in a safe way and achieves long lasting reductions of wrinkles.

Description

In the first aspect, there is provided a nanoemulsion, according to Claim 1.

A suitable nanoemulsion can be obtained with a botulinum protein-polymer conjugate. In nanoemulsions the emulsion droplets have an average droplet diameter of less than 1000 nm. Owing to their small average droplet diameter, they differ distinctly from conventional emulsions, which have average droplet diameters over 2000 nm.

The nanoemulsions can be produced according to known methods. The droplets in the nanoemulsion have an average diameter of at most 1000 nm, preferably less than 500 nm or less than 200 nm, and especially preferably approximately 100 nm.

PEG usually has an average molecular weight of 200 to 35000 Dalton. The name given to polyethylene glycols is determined by their average molecular weight. PEG-5000, for example, has an average molecular weight of 5000 Dalton. The duration of botulinum protein's effect in the body can be extended significantly by coupling it to PEG.

We have discovered that only botulinum protein-PEG conjugates containing medium-weight PEG have a longer-lasting effect in the body and are suitable for treating wrinkles. In the context of this application, we consider medium-weight PEG to be PEG with an average molecular weight of 2000 to 15000 Dalton, preferably 2000 to 10000 Dalton and especially preferably 5000 Dalton.

We also coupled the botulinum protein to other polymers, namely polyvinylpyrrolidone or hyaluronic acid. When selecting the conjugates for wrinkle treatment, it is essential that, in addition to having a significant wrinkle-reduction effect, the conjugates used are skin-friendly and do not cause any inflammation, pustules or peeling of the skin or trigger allergies. A slight redness which disappears by itself within a few hours after the application is, however, acceptable.

As illustrated in the Examples, the nanoemulsion of the invention achieve significant reduction in wrinkles and are skin friendly. This is due to the action of the PEG which allows the botulinum protein to pass directly across the skin. This effect is not taught by D1 or D2.

For a stable effect, it is advantageous if the droplets in the nanoemulsion are all the same size. It is also advantageous if there are no large droplets, e.g. with a diameter of more than 500 nm, in the nanoemulsion. Thus, preferably, the nanoemulsion droplets are as defined in Claim 2.

The skilled person knows that in a process for making nanoemulsions at least two immiscible phases are used, namely an aqueous and an oil phase. In our case, the aqueous phase can be, for example, water, a salt solution or an ethanol-water-mixture. Examples of oil phases are palm oil, almond oil, sunflower oil, soy oil, olive oil, silicon oil, avocado oil, or any other oil suitable for cosmetic use. The oil phase can also contain surfactants such as lecithin, phospholipids, polysorbate or stearylamine, lecithin being preferred because it is well tolerated. The botulinum protein or its conjugate are added to the aqueous phase.

To produce nanoemulsions, high shear forces must be generated. For this, known methods such as, for instance, high pressure homogenisation are used. In our firm, we use a microfluidiser as high pressure homogeniser. Obtaining a nanoemulsion by our method requires that the two phases are mixed and exposed to a pressure of at least 1000 bar for a period of at least 30 seconds to at most 10 minutes. It has proved advantageous to expose the two mixed phases to a pressure of 1500 to 2000 bar to obtain droplets having an average diameter of less than 500 nm.

When producing nanoemulsions, we noticed that the composition of the phases used for the method has an effect on the droplet size distribution. Two phases are mixed, the aqueous phase making up at least 90 vol. % and the oil phase 10 vol. % or less. The weight ratio of surfactant to oil is 2:1 or more, e.g. 2.5:1 or 3:1. If the mixed phases are now exposed to the advantageous high pressures mentioned above, droplets of the same size with a diameter of 100 nm +/- 10 nm are obtained.

Thus, preferably the nanoemulsion has droplets that are all the same size and have a diameter of 100 nm +/- 10 nm. These improve the stability of the effect observed; as shown in the examples.

In a second aspect, there is provided a method of making nanoemulsions as recited in Claim 4. Preferably, the pressure used in the process is 1500-2000 to provide droplets having 500 nm or less (see Claim 5).

Preferably, a surfactant is used (see Claims 6 and 7).

Preferably the method is as defined in Claim 8. This method advantageously allows droplets with a diameter of 100 nm +/- 10 nm to be formed which have greater stable effect, as shown in the accompanying examples.

In a third aspect, there is provided a cream for topical administration as defined in the Claim 9.

This cream is not disclosed or suggested by D1 which teaches away from using the botulinum protein conjugate in topical administration routes.

Preferably the cream can comprise the conjugate formulated as a nanoemulsion, as recited in Claim 10. The advantages of this cream is shown in examples.

All creams known to the skilled person can be used to produce the botulinum protein- containing cream. Other additives can be added to this cream, such as, for example, thickeners, colouring agents, fragrances, preservatives, or other substances which have the effect of smoothing out wrinkles such as vitamin C, retinol, collagen or coenzyme Q. Thus, there is provided a cream according to Claim 11.

In a fourth aspect, there is provided a method of reducing wrinkles by cosmetic application as recited in Claim 12.

The botulinum-protein-containing cream is generally applied every 1 to 4 months. If the wrinkling is moderate, it is recommended that it be applied at two-monthly intervals. In cases of heavy wrinkling, the cream can also be applied monthly. Thus, preferably the method is as defined in Claims 13 and 14.

In a fifth aspect, there is provided a kit as recited in Claim 15.

The botulinum protein conjugates and/or the nanoemulsion are stirred into a commercially available cream. This botulinum-protein-containing cream is best applied to the desired part of the face using a pipette in order to achieve a delivery of cream to the skin's surface as targeted as possible. Suitable pipettes are, for example, disposable pipettes made from polyethylene with a nominal volume of 1 to 10 ml and integrated suction bellows. Such disposable pipettes are sold by our subsidiary Easycare, Inc. under the brand name CANULETTA. Suitable pipettes can be sold together with our cream as a kit.

Examples

Not attached in accordance with candidates instructions.

Examination Committee I: Paper A Ch 2015 - Marking Details			Candidate No	
Category		Maximum possible	Marks awarded	
			Marker 004	Marker 001
Independent claims	Nanoemulsion comprising a	25	25	25
Independent claims	Cream comprising the	25	25	25
Independent claims	Method for cosmetic treatment;	20	16	16
Dependent claims	Dependent claims	15	11	9
Description	Description	15	14	14
Total			91	89
Examination Committee I agrees on 90 points and recommends the grade PASS				